Proceedings of the XIIth International Congress for Electron Microscopy

IMMUNOLOCALIZATION OF TYPE VI COLLAGEN IN CALFSKIN: A CORRELATIVE STUDY

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Although fine details of the fundamental structural units of calfskin, the types I and III collagen fibrils, have been well elucidated[1], little is known about the location of "minority" collagens in the skin before and after processing to leather. Using microscopic methods, we investigated one such collagen, type VI, in calfskin to determine its presence and distribution.

Four different methods of identification of type VI collagen were employed: immunofluorescence and peroxidase-antiperoxidase (PAP) staining using light microscopy, and transmission (TEM) and scanning (SEM) immunoelectron microscopy. In all cases, the primary antibody used was a polyclonal anti-type VI collagen raised in rabbits[5]. For light microscopy, 12 μm frozen sections were prepared. Fluorescent labeling was carried out with anti-rabbit IgG conjugated to r-phycoerythrin as secondary antibody. PAP staining was performed with the modified techniques of Amenta et al.[2]. Tissue for SEM was prepared by a modified protocol of Keene et al.[3] without prefixation: the secondary antibody used was goat anti-rabbit IgG conjugated to 30 nm colloidal gold[4]; it was then fixed in glutaraldehyde-paraformaldehyde and OsO_4, dehydrated, critical point dried and sputter coated with gold. Tissue for TEM was briefly fixed in 0.05% glutaraldehyde - 3.0% paraformaldehyde in 0.1M phosphate buffer, pH 7.1 before embedment in Lowicryl K4M; 60 nm sections were cut and mounted on coated nickel grids. The secondary antibody used was goat anti-rabbit IgG conjugated to 10 nm colloidal gold.

Fluorescent antibodies were found to be diffuse over the surface of the tissue sections, indicating the ubiquitous nature of the collagen. This was confirmed by PAP staining, in which the characteristic brown color was associated with the papillary and reticular dermis. In SEM images, gold particles associated with type VI collagen were located next to collagen bundles. TEM observations pinpointed type VI collagen in extracellular matrix at the peripheries of single type I/III collagen fibrils and fibril bundles. Using these four correlative methods, we identified and located type VI collagen. This information will be used to analyze the effects of type VI collagen on leather made from calfskin.

References

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4. deHarven, E., et al., 1987. Proc. 45th Ann. Mtg. EMSA. 45: 996-999.
5. Polyclonal antibodies to type VI collagen were courtesy of Dr. Eva Engvall.

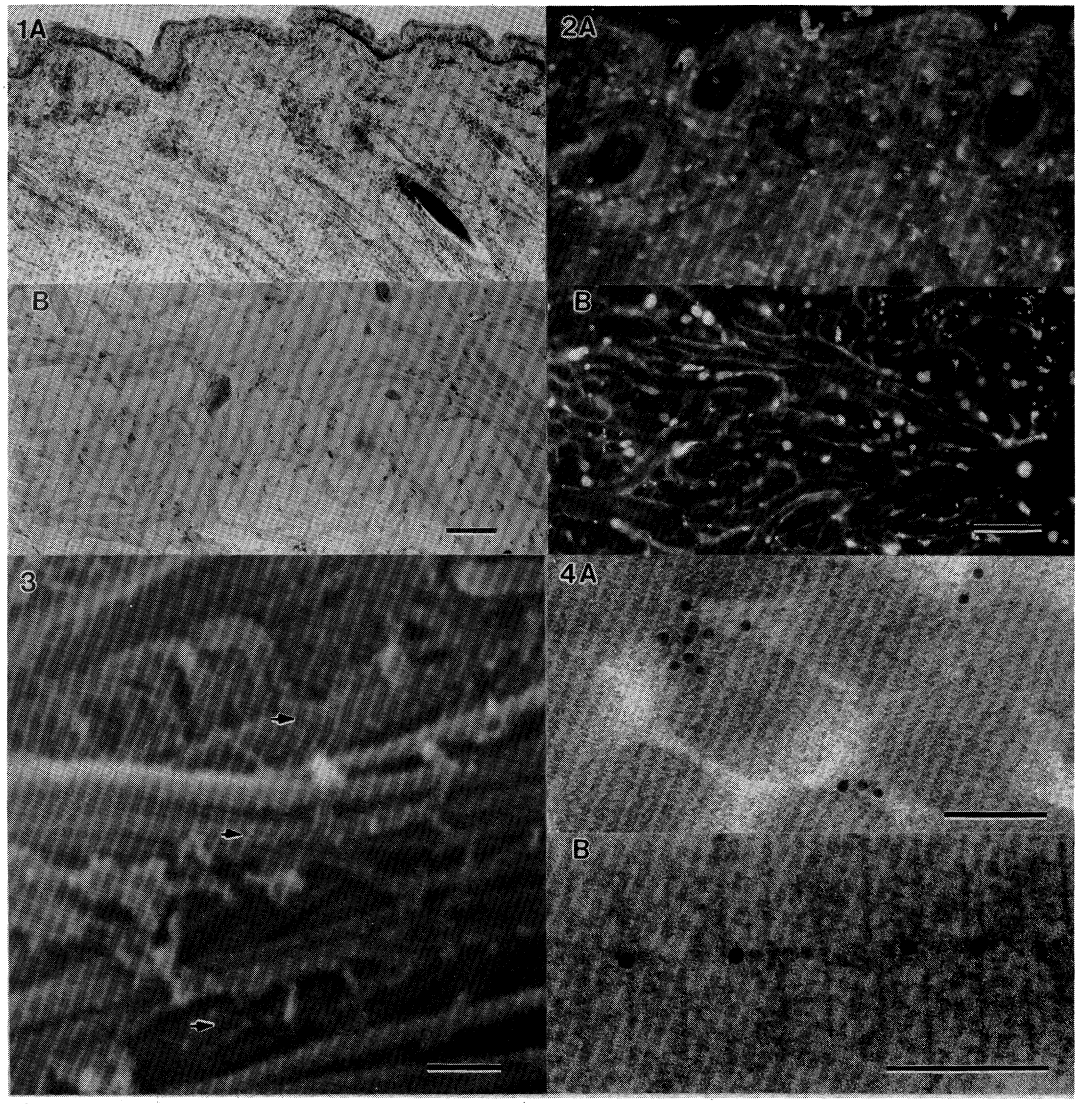

Fig. 1. Light micrographs of calfskin prior to leather processing. PAP reaction occurs over entire section.
a. papillary dermis (grain) with epithelium
b. reticular dermis (corium). Bar = 100 μm.

Fig. 2. Fluorescent antibody coupled to anti-type VI collagen. Fluorescence occurs with all cell types. Bar = 100 μm.

Fig. 3. Secondary electron image of 30 nm gold found peripheral to type I/III collagen fibrils, indicating presence of type VI collagen. Bar = 0.2 μm.

Fig. 4. Transmission electron images of 10 nm gold identifying location of type VI collagen in calfskin prior to leather processing.
a. Type VI collagen is found in extracellular matrix around cross-sectioned type I/III collagen.
b. Gold particles at interface between two longitudinal collagen fibrils. Bars = 0.1 μm.